International Journal of Basic and Applied Virology 10(2): 20-37, 2021
ISSN 2222-1298

DOI: 10.5829/idosi.ijbav.2021.20.37

A Review on Pathogenesis, Diagnostic Tests Used and Control Measures Undertaken for Rabies Virus: A Neglected Disease

Demeke Zewde

National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia

Abstract: Rabies is an acute encephalitis illness caused by rabies virus which is the type species under the family of *Rhabdoviridae*, genus lyssa virus and affects virtually all mammals. It is an enveloped RNA virus with negative-stranded genomes and non-segmented. Despite, rabies is a serious neglected tropical disease; it has the highest case fatality of any infectious disease particularly in warm-blooded animals including humans. Due to its perpetuation in the wildlife rabies is not eradicable, but is preventable and controllable. The aim of this paper is to review the pathogenesis, diagnostic tests used and control measures are undertaken and to recommend for further research that able to tackle the severity of the disease. Rabies is endemic in the developing countries of Africa and Asia, which also leads to death once clinical signs are, manifested. In spite of the long history of rabies, its pathogenic process remains poorly understood. Most of what we know about the disease process is acquired from investigations conducted in experimental animal models. The overall outcome of an exposure to RABV (rabies virus) depends in part upon the rabies genotype or variant involved, its pathogenicity, the dose of virus inoculated, the route as well as the host species and its susceptibility to the particular pathogen together with innate and adaptive immune responses of the host. Demonstration of Negri bodies by direct fluorescent antibody test (FAT), latex agglutination test, Virus isolation in new born mice, virus isolation in cell cultures, avidin-biotin test, ELISA (Enzyme linked immune-sorbent assay), Electron Microscopy and recently molecular methods have been used to detect rabies antigen. Since their development more than four decades ago, concentrated, purified cell culture and embryonated egg-based rabies vaccines (CCEEVs) have proved to be safe and effective in preventing rabies in humans. This review describes in detail pathogenesis, diagnosis and control strategies on rabies.

Key words: Control · Diagnosis · Neglected Disease · Pathogenesis · Rabies · Review

INTRODUCTION

Rabies is a fatal zoonotic disease that causes encephalitis in all warm blooded animals and humans [1]. There have been indications about the occurrence of rabies from the time of Homer onwards and it is originated about 3000 B.C. Rabies is an acute encephalitis illness caused by rabies virus. Rabies virus is the type species family of *Rhabdoviridae*, which affects virtually all mammals [2]. Rabies is endemic in the developing countries of Africa and Asia, which also leads to death once clinical signs are, manifested [3]. Rabies is a preventable viral disease of mammals most often transmitted through bite of rabid animals. It is prevented by eliminating rabies through animal's vaccinations as well as by preventing entry and contact of infected dogs and wild animals with domesticated animals [2].

Rabies virus infects a wide range of hosts, including dogs, cats, raccoons, skunks, foxes, coyotes, bats and human beings [4]. RABV is usually transmitted to humans through a bite from domestic or wild animals. It invades the central nervous system (CNS) which leads to acute encephalitis and death [4, 5]. It has been estimated that about 70, 000 people die from rabies each year, mostly in Africa and Asia [6].

In the world, it has been estimated that about 70, 000 people die from rabies each year, of which the highest death is in Asia and Africa [6]. Canine rabies

Corresponding Author: Demeke Zewde, National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia. Tel: +251917839589.

continued to be a serious problem in Africa, including Ethiopia [7]. Dogs are the principal source of infection (99%) for humans and livestock [8]. Rabies has also significant economic importance by its effect on livestock. For example, in Africa and Asia, the annual cost of livestock losses as a result of rabies is estimated to be US$ 12.3Million [9].

The incubation or eclipse period of rabies is highly variable from 2 weeks to 6 years (avg. 2–3 months), which entirely depends on the concentration of the virus inoculated, inoculation site and density of innervations [10]. The greatest risk factor is bites on the hands, neck, face and head mainly with bleeding lead to shorter incubation period due to the decreased length and greater number of neurons. Generally RABV can persist in the muscle for prolonged duration, which may give a chance for post-exposure treatment and clearance of RABV by the immune system of the host [11].

In spite of significant advancement in diagnosis, control and prevention of rabies, its pathogenesis especially in rodents using fixed strains is not clearly understood [12, 13]. Therefore, the aim of this review is to assess the nature and causative property of the virus, to highlight approaches of diagnosis and ways of control and prevention methods applied for the disease.

Viral Characteristics

Classification and Taxonomy of Rabies Virus: The rabies virus genome consists of a single stranded, nonsegmented, enveloped, negative sense RNA of approximately 12kb [14]. It is under Lyssavirus genus, in the family of Rhabdoviridae, order Mononegavirales which is transmissible to all mammals [15]. Seven distinct genetic lineages can be distinguished within the genus *Lyssavirus* by cross-protection tests and molecular biological analysis [16, 17], namely the classical rabies virus itself (RABV, genotype 1, serotype 1), Lagos bat virus (LBV, genotype 2, serotype 2), Mokola virus (MOKV, genotype 3, serotype 3) and Duvenhage virus (DUVV, genotype 4, serotype 4). The European bat lyssaviruses (EBLV), subdivided into two biotypes (EBLV1, genotype 5 and EBLV2, genotype 6) and the Australian bat lyssavirus (ABLV, genotype 7), isolated in Australia [18], are also members of the *Lyssavirus* genus, but are not yet classified into serotypes. Also four putative viruses (Aravan, Khujand, Irkut and West Caucasian Bat Virus, Shimoni bat virus isolated in 2009) as well as Bokeloh bat lyssavirus [19] and the Ikoma lyssavrus [20] were discovered in 2011 and 2012 respectively. Viruses of serotypes 2–4, EBLV and ABLV are known as rabies-related viruses.

Viral Structure/ Morphology: Rabies viruses have approximately 180 nm long and 75 nm wide [21] and its genetic information is organized in the form of a helical ribonucleoprotein complex (RNP), in which the linear RNA is tightly associated with the viral nucleoprotein (Figure 1). The genome of RABV encodes for only five proteins in the order: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the large protein (L, also termed RNA-dependent RNA polymerase, RdRp) [22] (Figure 2). The N plays a critical role in viral transcription and replication [23]. The G forms approximately 400 trimeric spikes, which are tightly arranged on the surface of the virions [24]. The G is a major determinant for RABV neuropathogenicity by binding specific receptor(s), entering the nervous system through the endosomal transport pathway via a low-pH-induced membrane fusion process [25].

The N-L-P polymerase complex starts transcription with the neither production of a short RNA molecule, the leader RNA, that is neither capped nor polyadenylated. Subsequently, mRNAs are produced for N, P, M, G and L. The switch between transcription and replication of genomic RNAs are controlled by the level of N protein [27]. All transcription and replication events take place in the cytoplasm inside a specialized ‘virus factory’, the Negri body [28].

Epidemiology of the Virus

Distribution and Animal Reservoir of Rabies: Although all mammals are susceptible to RABV, only a few species are important as reservoirs for the disease. Dogs remain the most important reservoirs for rabies in the developing countries of Asia and Africa [29]. Rabies is still present in Europe, but the human rabies has been disappeared from many European countries. The disappearance of rabies was probably due to enforced policy of animal vaccination. The epidemiological and genetic analysis of many isolates showed that canine rabies remains in certain countries as well as borders of Europe [30].

Few countries Great Britain, New Zealand, Australia and Iceland claim to be free of disease due to their island status, successful elimination programs and enforcement of rigorous quarantine regulations [31-34].

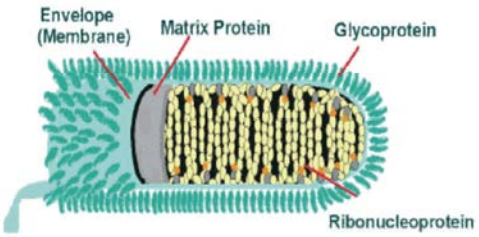

Fig. 1: A bullet shape of rabies virus

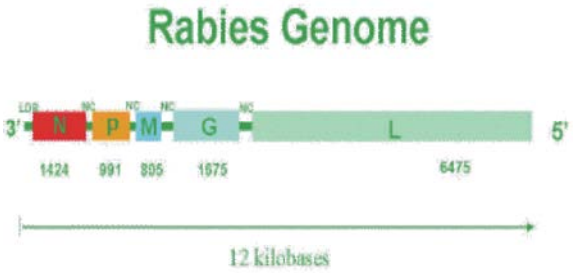

Fig. 2: Genomic structure of rabies virus
Source: [26]

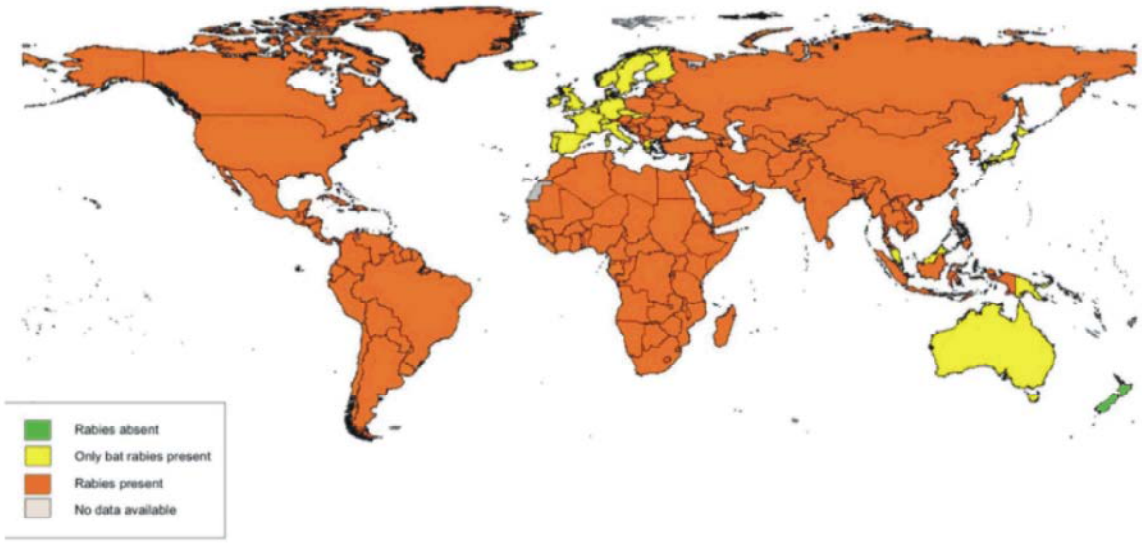

Fig. 3: Distribution of rabies in the world, based on the data from WHO (2007)
Source: [44]

With the exception in Australia where other types of zoonotic lyssaviruses transmitted by flying bats/fox, the disease is endemic in mammals and other warm-blooded vertebrates [35]. To date, in Australia RABV does not occur in land dwelling animals. However, closely related viruses but not identical to RABV like ABLV often present in Australia and can be transmitted from bats to humans and animals [36, 37].

In the developed nations, dog rabies has been eliminated or controlled through mass vaccination during the past 70 years [38]. However, wildlife rabies becomes a major concern. In North America, rabies is endemic in raccoons, foxes, coyotes and skunks [38] while fox rabies is endemic in Europe [39]. Wolves, jackals and other wild animal species have also been reported as reservoirs in other regions [40]. Bats are probably the ultimate reservoirs for rabies virus (Figure 3) [41]. In the Americas, a number of bat species carry distinct rabies virus strains [42], whereas in Europe and Australia bats carry rabies-related lyssaviruses [29].

Canine rabies has been eliminated from Western Europe, Canada, the United States of America (USA), Japan, Malaysia and a few Latin American countries; while Australia is free from carnivore rabies and many Pacific island nations have always been free from rabies and related viruses. In these areas, human deaths from rabies are restricted to people exposed to while living or travelling in areas endemic for canine rabies. About two deaths per year due to imported human rabies have been reported in Europe, North America and Japan [43].

Pathogenesis of Rabies: Despite the long history of rabies, its pathogenic process remains poorly understood. Most of what we know about the disease process is acquired from investigations conducted in experimental animal models. The overall outcome of an exposure to RABV depends in part upon the rabies genotype (different strains and mutants) or variant involved, its pathogenicity (apoptogenicity, neuroinvasiveness), the dose of virus inoculated (severity of exposure), the route as well as the host species and its susceptibility to the particular pathogen together with innate and adaptive immune responses of the host [45]. However, various studies in animal models indicate that the pathogenic wild-type/street RABV and the fixed (laboratory-adapted) RABV evidently behave differently in each step of their life cycle in the host.

RABV G is the only surface protein of the virion and capable of inducing virus neutralizing antibodies (VNA) [46]. The G protein plays an important role in rabies pathogenesis [47] by binding to neural receptor such as acetylcholine receptor [48] and neural cell adhesion molecules (NCAM) [49] contributing to the exclusive neurotropism and neuroinvasiveness of RABV [50]. Virus may enter muscles and replicate at the site of inoculation or enter directly into peripheral nerves without prior replication in non-neural tissues [51]. It is believed that once virus particles enter the peripheral nervous system and start to spread to the Central nervous system (CNS), a fatal outcome of the disease is inevitable, though there are some reports of rabies survivors. RABV enters motor and/or sensory axons of peripheral nervous system and spreads to the CNS by retrograde fast axonal transport at a rate of approximately 50–200 mm/day [52].

The pathogenic RABV have evolved specific mechanisms to escape early immune system recognition in the periphery via limited replication, minimized G expression [53] suppression of interferon response, anti-apoptotic stimulation and transportation through neurons only. On the other hand, fixed RABV induces extensive inflammation by activating innate immune responses [53], induces apoptosis [54], replicates to higher levels and express high levels of the G protein [55]. However, the mechanism adopted by the fixed RABVs to elicit immune responses and the wild-type RABVs to evade immune system is still not entirely clear. It has been shown that the innate immune responses and inflammation in the CNS is associated with BBB permeability enhancement [56] in mice infected with fixed RABV but not in those infected with street RABV [57]. Current understanding on the striking difference between pathogenic and nonpathogenic rabies biology is summarized (Table 1).

Until recently, our knowledge of RABV pathogenesis was limited and largely based on descriptive studies with street RABV strains or experimental infection with attenuated laboratory-adapted strains. The advent of reverse genetics technology made it possible to identify viral elements that determine the pathogenic phenotype of RABV and to obtain a better insight into the mechanisms involved in the pathogenesis of rabies [65].

Viral Elements Involved in Virus Uptake: After receptor binding, rabies virus is internalized via adsorptive or receptor-mediated endocytosis [66]. Then, the low pH environment within the endosomal compartment causes a conformational change in RABV G, which triggers the fusion of the viral membrane to the endosomal membrane, thereby releasing the RNP into the cytoplasm [67]. It has been demonstrated that the pathogenicity of tissue culture-adapted RABV strains like Evelyn Rokitnicki Abelseth (ERA) correlates with the presence of antigenic determinant located in antigenic site III of the G protein [65]. An Arg→Gln mutation at position 333 in this antigenic site of the ERA G protein resulted in a sevenfold delay of internalization of the Gln333 RABV variant as compared with that of the wild-type.

An Asn_{194}→Lys_{194} mutation in RABV G, which accounted for the re-emergence of the pathogenic phenotype, was associated with a significant decrease in the internalization time [68]. The time necessary for internalization of RABV virion was significantly increased and the pathogenicity was strongly reduced after the exchange of the G gene of the highly pathogenic RABV strain Sylvatic bat (SB), which was derived from a cDNA clone obtained from the silver-haired bat-associated RABV-18 strain [69], with that of a highly attenuated SN strain, which was rescued from a cDNA clone of the Street-Alabama-Dufferin (SAD B19) RABV vaccine strain [70]. These support the notion that the kinetics of virus uptake, which is a function of RABV G, is a major factor that determines the pathogenicity of RABV.

Table 1: Striking difference between pathogenic and non-pathogenic RABV biology

Parameters	Non-pathogenic virus (fixed/lab-adapted)	Pathogenic virus (street/wild-type)	References
Cellular Tropism	Not exclusively neuronal	Highly neuronal	50; 58]
Glycoprotein Expression Levels	High	Low	[55; 58]
Replication (titer)	High	Low	[50]
Apoptosis	High	Low	[58; 59]
Interferon sensitivity	Resistant	Highly sensitive	[60]
Immune System	Activates innate/adaptive immunity	Evades innate/adaptive immunity	[61; 62]
Blood-brain-barrier (BBB) permeability	Enhances	Little or no change	[63; 64]

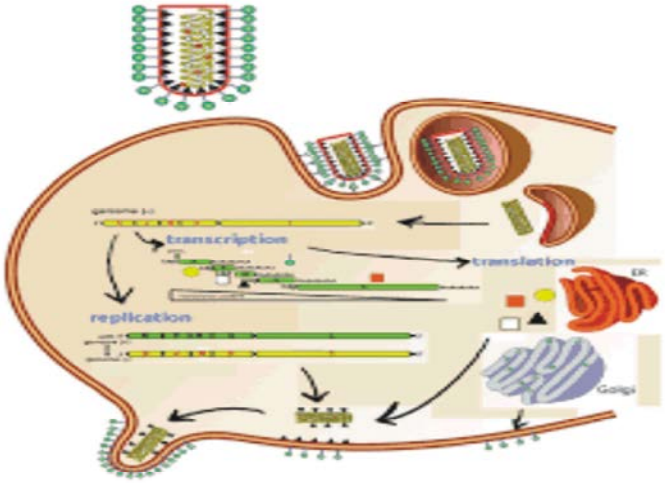

Fig. 4: The spread and evolution of rabies virus
Source: [78]

Viral Elements Involved in Virus Spread and Transport: A unique property of RABV is its ability to spread from cell-to-cell and it is transported intact within endosomes, but the significance of this transport for productive infection has not been examined [71]. The observation that the Gln333 ERA variant loses pH-dependent cell fusion activity *in vitro* [72] and exhibits a strongly reduced ability to spread from cell to cell [73] suggests that RABV G also plays a pivotal role in cell-to-cell spread and thus viral transport, probably through its fusiogenic activity.

The finding that viral spread of the ERA G $Arg_{333}\rightarrow Gln_{333}$ mutant within the CNS is strongly reduced as compared with that of the wild-type [72], also points to the function of intact RABV G in trans-synaptic spread. The strongest evidence for an essential role of RABV G in trans-synaptic transport, however, comes from intracranial infection of mice with a RABV G-deficient recombinant virus, which showed that the infection remained restricted to neurons at the inoculation site without any signs of spread to secondary neurons [74].

In addition to RABV G, RABV M also plays a role in virus spread and thus in trans-synaptic transport. In this respect it was shown that spread of the chimeric RABV SN-BMBG variant, which contains both M and G from the highly pathogenic SB, was significantly higher than that of the chimeric SN-BG or SN-BM, which contain G and M from SB, respectively, suggesting that an optimal interaction of M with G might play an important role in virus cell-to-cell spread [75]. Since RABV M supports virus budding [76], it is likely that the more efficient spread of the chimeric RABV SN-BMBG variant is due to optimal virus budding at the postsynaptic membrane. Peripheral infection of adult mice showed that removal of the LC8 binding domain from RABV P does not prevent virus entry into the CNS suggesting that the RABV protein is not directly involved in the retrograde axonal spread of RABV [77].

Viral Elements That Control Virus Replication: Unlike many other viruses, such as influenza virus, the pathogenicity of RABVs correlates inversely with the rate of viral RNA synthesis and the production of infectious virus particles. The M of all *rhabdoviruses* is able to shut down viral gene expression by binding to the RNP, resulting in a highly condensed skeleton-like structure that is unable to support RNA synthesis [76].

To identify other viral elements that control pathogenicity by regulating virus replication, 5'-end sequences of the highly pathogenic SB strain were exchanged in a stepwise manner with those of the highly attenuated vaccine strain SN, resulting in the recombinant viruses SB2 (trailer sequence [TS] + L), SB3 (TS + L + pseudogene [Ø]), SB4 (TS + L + Ø + G) and SB5 (TS + L + Ø + G + M) [69]. Intramuscular infection with parental viruses SB and SN and with the chimeric RABVs SB2, SB3, SB4 and SB5, revealed the highest mortality rates in mice infected with SB and no morbidity or mortality in mice infected with SN. Replacement of TS, L and Ø in SB with the corresponding elements from SN resulted in a moderate decrease in morbidity and mortality and additional exchange of G or G plus M strongly reduced or completely abolished virus pathogenicity. Additional evidence for an inverse correlation between pathogenicity and the rate of viral RNA synthesis and virus particle production was obtained from mice infected with chimeric recombinant viruses in which the G and M genes of the attenuated SN strain were exchanged with those of the highly pathogenic SB strain [75]. These experiments revealed a significant increase in the pathogenicity of the SN parental strain bearing RABV G from the pathogenic SB strain. These data indicate that both G and M play an important role in RABV pathogenesis by regulating virus replication. The finding that replacement of G or G plus M in SN by the G or G plus M of SB results in a moderate or strong decrease in viral RNA transcription and replication, respectively, while replacement of only the M in SN with the SB M results in a strong increase in viral RNA transcription and replication, indicating that RABV G also has an important regulatory function in viral RNA transcription/replication, either alone or via interaction with the M protein. The mechanism by which the RABV G gene controls viral RNA synthesis is not known [75].

To evade the immune response and to preserve integrity of the neuronal network, pathogenic RABV strains, but not attenuated strains, can regulate their growth rate. A lower replication level probably benefits the pathogenic RABV strains by conserving the structure of neurons that are used by these viruses to reach the CNS. Another explanation for the lower replication rate of pathogenic RABVs is that in order to evade early detection by the host immune system, the virus keeps the expression levels of its antigens at a minimum [53].

Diagnostic Techniques: Several techniques such as; demonstration of Negri bodies by direct fluorescent antibody test (FAT), latex agglutination test, Virus isolation in new born mice, virus isolation in cell cultures, avidin-biotin test, ELISA, Electron Microscopy and recently molecular methods have been used to detect rabies antigen details of which have been published in many review articles[79]. However, FAT is gold standard recommended by both WHO and OIE and the most widely used test for rabies diagnosis as it is highly sensitive, specific, cheap and gives reliable results providing results within few hours in more than 95-99% of rabies cases [80]. Despite the availability of vaccines to prevent this disease, it is still a significant public and veterinary health problem in many countries particularly in Asia and Africa [9, 81, 82] as a result of lack of accurate data on the true impact of the disease and lack of political commitment for its control [9].

Collection of Samples: In the brain, rabies virus is particularly abundant in the thalamus, pons and medulla. The hippocampus (Ammon's horn), cerebellum and different parts of the cerebrum have been reported to be negative in 3.9–11.1% of the positive brains. The structure of choice is the thalamus as it was positive in all cases. It is recommended that a pool of brain tissues that includes the brain stem should be collected and tested [83]. To reach these parts of the brain, it is necessary to remove the entire organ after having opened the skull in a necropsy room. Under some conditions a simplified method of sampling through the occipital foramen [84], or through the orbital cavity [85], can be used. Personal protection is mandatory when handling central nervous system tissues from suspected rabies cases.

Occipital Foramen Route for Brain Sampling: A 5 mm drinking straw [84] or a 2 ml disposable plastic pipette [86] is introduced into the occipital foramen in the direction of an eye. Samples can be collected from the rachidian bulb, the base of the cerebellum, hippocampus, cortex and medulla oblongata. Bovine spongiform encephalopathy (BSE) should be considered in the differential diagnosis of most cattle that are considered to be 'rabies suspect'.

Retro-Orbital Route for Brain Sampling: In this technique [85], a trocar is used to make a hole in the posterior wall of the eye socket and a plastic pipette is then introduced through this hole. The sampled parts of the brain are the same as in the former technique, but they are taken in the opposite direction.

Shipment of Samples: As rabies virus is rapidly inactivated, refrigerated diagnostic specimens should be sent to the laboratory by the fastest means available. Shipment conditions must be considered to be part of the

'rabies diagnostic chain'. During the shipment of suspect material for diagnosis (animal heads, brain or other tissue samples), no risk of human contamination should arise. Brains must be placed in a leak-proof rigid container (animal heads will be wrapped in absorbent material) as prescribed in the International Air Transport Association (IATA) Dangerous Goods Regulations must be followed. When it is not possible to send refrigerated samples, other preservation techniques may be used. The choice of the preservative is closely linked to the tests to be used for diagnosis. Formalin inactivates the virus, thus the isolation tests cannot be used and diagnosis depends on using a modified and less sensitive direct fluorescent antibody test (FAT), immunohistochemistry or histology [15].

Infectivity at room temperature may be extended for several days if brain material is kept in a mixture of 50% glycerol in phosphate buffered saline (PBS). Glycerol/PBS slows bacterial action and therefore protects against the chemical and biological effects of putrefaction. It does not protect against titre decline due to thermal conditions and therefore, because rabies is thermo-labile, the virus titre will decline during glycerol/PBS storage. Therefore, whenever possible samples in glycerol/saline should be kept refrigerated; as the virus is not inactivated by glycerol/PBS [15].

Identification of the Agent

Based on Clinical Signs and History: The clinical signs of rabies are confused with other neurological sings caused by other neurotropic etiological agents. Rabies infection has a variable and lengthy incubation period in humans and animals generally last up to 20 to 90 days [87; 88]. In 75% of dogs, the early signs (2–5 days duration) progress to paralytic or dumb forms. This is very risky while attending the dumb form of cases. Paralysis as well as mortality happens in both clinical forms at 4 to 8 days after the appearance of clinical signs. Based on some typical clinical sings in dogs and other animal species, a tentative diagnosis can be made [89]. The animals showing abnormal behavior should be kept in isolation where they cannot bite others for 10 days. Marked changes in behavior are seen in the prodromal phase of rabies. They vary with the species like irritable, increased sensitivity to noise and light, more alert, restless and friendly, aggressive (more in cat) and attack without provocation, or become depressed, hiding in dark places, slight pyrexia, impaired corneal reflex and self-mutilation at the site of the bite. In excitative phase, nervous signs like irritability, vicious bite and attack, muscle tremors, flaccidity or in-coordination, pica, spasm and paralysis of deglutination, change in voice, difficulty in swallowing, drooling as well as frothing of saliva, dropping of jaw, paralysis, coma and death. No specific lesions are observed grossly in the brain. However, wound due to the bite and presence of foreign bodies in stomach due to pica may be suspected for rabies. The disease should be differentiated from canine distemper, canine, equine and bovine encephalitis, hepatic encephalopathy, thiamine deficiency (cats), poisoning due to lead and organochloride compounds, benzoic acid, strychnine poisoning, pseudorabies, spongiform encephalopathy and listeriosis [16, 90, 91]. Bat-acquired rabies may present with different clinical manifestations than dog-acquired rabies. These findings may help in improving the early diagnosis of rabies [92]. The only way to perform a reliable diagnosis of rabies is to identify the virus or some of its specific components using laboratory tests.

Fluorescent Antibody Test (FAT): The most widely used test for rabies diagnosis is the FAT, which is recommended by both WHO and OIE. This test may be used directly on a smear and can also be used to confirm the presence of rabies antigen in cell culture or in brain tissue of mice that have been inoculated for diagnosis. The FAT gives reliable results on fresh specimens within a few hours in more than 95–99% of cases. The sensitivity of the FAT depends on the specimen (the degree of autolysis and how comprehensively the brain is sampled,) [93], on the type of lyssavirus and on the proficiency of the diagnostic staff. Sensitivity may be lower in samples from vaccinated animals due to localization of antigen, which is confined to the brainstem. For direct rabies diagnosis, smears prepared from a composite sample of brain tissue that includes the brain stem, are fixed in high-grade cold acetone and then stained with a drop of the specific conjugate. Anti-rabies fluorescent conjugates may be prepared in the laboratory. In the FAT, the specific aggregates of nucleocapsid protein are identified by their fluorescence (Figure 5).

The FAT may be applied to glycerol-preserved specimens. If the specimen has been preserved in a formalin solution, the FAT may be used only after the specimen has been treated with a proteolytic enzyme [95, 96]. However, the FAT on formalin-fixed and digested samples is always less reliable and more cumbersome than when performed on fresh tissue [96].

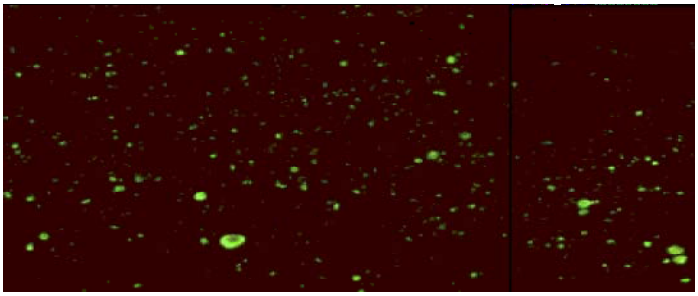

Fig. 5: Microscope image of nervous tissue with positive direct FAT
Source: [94]

Detection of the Replication of Rabies Virus after Inoculation: These tests detect the infectivity of a tissue suspension in cell cultures or in laboratory animals. They should be used if the FAT gives an uncertain result or when the FAT is negative in the case of known exposure [15].

Cell Culture Test: Neuroblastoma cell lines, e.g. CCL-131 in the American Type Culture Collection (ATCC) 1, is used for routine diagnosis of rabies. The cells are grown in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal calf serum (FCS), incubated at 36°C with 5% CO2. Its sensitivity has been compared with that of baby hamster kidney (BHK-21) cells [97]. This cell line is sensitive to street isolates without any adaptation step, but should be checked for susceptibility to locally predominant virus variants before use. Presence of rabies virus in the cells is revealed by the FAT. The result of the test is obtained after at least 18 hours (one replication cycle of virus in the cells); generally incubation continues for 48 hours [98] or in some laboratories up to 4 days. This test is as sensitive as the mouse inoculation test. Once a cell culture unit exists in the laboratory, this test should replace the mouse inoculation test as it avoids the use of live animals, is less expensive and gives more rapid results. It is often advisable to carry out more than one type of test on each sample, particularly when there has been human exposure [98].

Mouse Inoculation Test: Five-to-ten mice, 3-4 weeks old (12-14 g), or a litter of 2-day-old newborn mice, are inoculated intracerebrally. The inoculum is the clarified supernatant of a 20% (w/v) homogenate of brain material (cortex, Ammon's horn, cerebellum, medulla oblongata) in an isotonic buffered solution containing antibiotics. To reduce animal pain, mice should be anaesthetised when inoculated. The young adult mice are observed daily for 28 days and every dead mouse is examined for rabies using the FAT. For faster results in newborn mice, it is possible to check one baby mouse by FAT on days 5, 7, 9 and 11 post-inoculation. Any deaths occurring during the first 4 days are regarded as nonspecific (due to stress/bacterial infection etc.). This *in-vivo* test should be avoided when possible on animal welfare grounds. It is also expensive, particularly if SPF mice are used and does not give rapid results (compared with *in-vitro* inoculation tests), but when the test is positive, a large amount of virus can be isolated from a single mouse brain for strain identification purposes [99].

Histological Identification of Characteristic Cell Lesions: Negri bodies correspond to the aggregation of viral proteins, but the classical staining techniques detect only an affinity of these structures for acidophilic stains. Immunohistochemical tests are the only histological test specific to rabies. An unfixed tissue smear may be stained by the Seller's method; diagnosis is then obtained in under 1 hour. Histological tests, such as Mann's test, are performed on fixed material after a paraffin-embedding step and the result of the test is obtained within 3 days. These techniques have the advantage that the laboratory equipment needed to perform them is inexpensive and any need to keep specimens cold after fixation is avoided. These histological methods, especially the Seller's method, can no longer be recommended because they have very low sensitivity and should be abandoned [21].

Other Identification Tests

Enzyme-Linked Immune Sorbent Assay (ELISA): Commercial kits are available for indirect ELISA that allow a qualitative detection of rabies antibodies in individual dog and cat serum samples following vaccination. In accordance with the WHO recommendations [100], 0.5 IU per ml rabies antibodies is

the minimum measurable antibody titre considered to represent a level of immunity that correlates with the ability to protect against rabies infection. The ELISA provides a rapid (~ 4 hours) test that does not require handling of live rabies virus, to determine if vaccinated dogs and cats have sero-converted. The sensitivity and specificity of any kit used should be determined by comparison with virus neutralization methods. The ELISA is acceptable as a Prescribed Test for international movement of dogs or cats provided that a kit is used that has been validated and adopted on the OIE Register as fit for such purposes [101].

Detection of Viral Nucleic Acid: Several nucleic acid (NA) based tests (*in situ* hybridization, PCR, genomic sequencing, etc.) have been developed, which are in use (WHO or OIE reference laboratories) for diagnosis of rabies [102, 103]. These methods detect highly specific molecular subunits of rabies RNA in brain material either in experimental or in routine specimens. PCR based tests are also used for studying viral pathogenesis and epidemiological work apart from diagnosis. PCR can easily detecting few numbers of viral particles in a sample. To carry out PCR nucleotide sequence the target genome of the (viral) pathogen must be known. The PCR coupled with sequencing of amplified product can further provide accurate genotyping of RABV.

A simplified version of hemi-nested RT-PCR (hnRT-PCR) has been developed for determination of nucleoprotein gene of RABV specifically by Picard Meyer *et al.* [104]. Determination of the specificity of this method has been done by the use of seven genotypes of lyssavirus by RT-PCR along with Southern blot and sequence analysis. A higher sensitivity is shown by hnRT-PCR in order to detect small amounts of RABV nucleoprotein gene in comparison to the other diagnostic methods. This new hnRT-PCR is found to be an important diagnostic tool in the context of epidemiological investigation in relation to rabies. Alternative use of hnRT-PCR assay has been recommended in laboratories without having access to real-time-PCR equipments, which are expensive [105].

RT-PCR–ELISA has been developed for the diagnosis of RABV [106]. Diagnosis of rabies has been carried out by RT-PCR assay on brain samples obtained from different species of animals after postmortem [107]. Ante-mortem and post-mortem rabies diagnosis by RT-PCR has been demonstrated [108]. TaqMan real time-PCR was found to be quite sensitive for the diagnosis of rabies from body secretion/excretion [109, 110]. Absence of vaccine-associated rabies cases following oral vaccination was demonstrated with the help of pyrosequencing of the RVG gene [111].

DNA microarray has been used as a diagnostic tool for detection of nucleic acid segment of lyssaviruses including RABV [112]. The tailored microarray has got the capability of detecting as well as distinguishing all the seven recognised members of the genus *Lyssavirus* by use of 624 70 mer probes, both in humans and animals. The target for the microarray is the viral N gene because of the lower degrees of conservation among the seven genotypes of the lyssavirus. It further enables to generate species-specific probes. For detection as well as classification of isolates of lyssavirus into their respective genotypes, extensive use of the N gene has been done [113].

Dupuis *et al.* [114] compared the quantitative RT-PCR (qRT-PCR) technique with the gold standard dFAT and results were comparable between the both techniques, with one extra sample diagnosed by qRT-PCR when compared to dFAT. [115] validated a two-step lyssavirus qRT-PCR for the diagnosis of various lyssavirus species using degenerate primers and the assay was highly sensitive, specific and reproducible. The specificity was 100% and the sensitivity was superior when compared to dFAT.

Control and Prevention of Rabies

Pre-Exposure Vaccination: In modern days, the key stone to prevent and control of rabies is management of animals [33, 35, 116]. On primary ground, vaccination of dogs and cats alongside eliminating stray animals and public health education, etc., are the components of animal rabies control. Preventive immunization is suggested by the WHO for all the staffs involved in the handling of materials that are infected or suspected. Three injections are included in the immunization protocol at 0, 7 and 28 days. Serological estimation of antibodies should be done 1 to 3 weeks after the last immunization. Re-examination should be done in every 6 months for persons working in laboratory or every 2 years for other diagnosticians. Even if the titre goes below 0.5 International Units (IU) per ml, booster vaccination is recommended for sure. When serological monitoring is not available, booster vaccination is recommended at 1 year followed by vaccination at every 1-3 years [117, 118]. Because of the zoonotic significance veterinary technicians, veterinarians and other animal health workers should be vaccinated against rabies [35, 119, 120].

For animal use, rabies vaccines consist of live attenuated virus (e.g. Flury high egg passage, Flury low egg passage, Street-Alabama-Dufferin and Kelev), chemically or physically inactivated virus and recombinant vaccines. Embryonated eggs, CNS tissues from newborn animals and cell cultures are preferred for cultivation of virus [121]. Rabies vaccines are usually lyophilized but inactivated with an adjuvant and storage can be done in liquid form. Recently, for management of rabies in free-ranging bats, modified-attenuated vaccinia Ankara (MVA) and raccoon poxvirus (RCN) vaccine vectors were administered in the Brazilian free-tailed bat (*Tadarida brasiliensis*) by oronasal and intramuscular routes [122]. Significant levels of neutralizing antibody titers against rabies were identified in the serum of immunized bats. These studies highlighted the safety and immunogenicity of attenuated poxviruses and their potential use as vaccine vectors in bats. Immunization of domestic cattle and two-humped camel with canine inactivated rabies vaccine in Ningxia Hui and Inner Mongolia Autonomous Region of northwest China provides protection against rabies for at least one year [123].

The development of vaccines has been influenced by the advent of genetic engineering that provides more opportunities to produce inactivated antigens and to attenuate different viruses through direct mutation [124]. Various novel approaches like application of vectors, lipopeptide vaccines, plasmid DNA and plant virus-based rabies vaccines are also being explored [125]. Also, the paucity of vaccine adjutants, essentially limited to aluminum salts, is corrected at last by producing new emulsions (oil-in-water), toll-like receptor agonists, liposomes, cytokines, cpG oligonucleotides and other materials [126, 127].

There are limitations of applying vaccine preparations that are injectable especially in carnivores and wildlife. Oral vaccine formulation, therefore, has been attempted continuously with significant results in laboratory as well as field trials [128, 129]. Oral vaccines are mainly based on the avirulent mutants or the use of recombinant vaccines [128, 130].

The efficacy of killed vaccinia rabies-glycoprotein recombinant vaccines is also proven. The preparations of these vaccines involve insertion of non-infectious nucleic acid of RABV into a vector viz., vaccinia or canary pox [124]. It is important to note that there must not be any restriction over the entry of animals vaccinated with rabies-glycoprotein recombinant vaccines into any country since these vaccines do not contain live virus [131, 132]. A recombinant RABV containing two copies of G gene has been reported to protect dogs against a virulent challenge [133]. Inactivated vaccine consisting of recombinant rCVS-11-G strain encoding two copies of G protein from the pathogenic wild-type CVS-11 strain can act as a promising vaccine candidate for its enhanced immunogenicity [134].

The advantages and limitations of intra-dermal pre-exposure rabies vaccination have been reported [135]. The worldwide incidence of rabies and the inability of currently used vaccination strategies to provide highly potent and cost-effective therapy indicate the need for alternate control strategies [136, 137]. A replication-deficient RABV-based vaccine in which the matrix gene is deleted (RABV-ΔM) was shown to be safe and induced rapid and potent virus neutralizing antibodies [138]. The role of CD4+ T cell-independent B cell, IgM and IL-21 in the vaccine induced-immunity has been defined [139].

Post-Exposure Management: It is mandatory to strictly follow quarantine for 6 months for any animal exposed to a confirmed or suspected rabid animal. Upon entry into isolation or 1 month before release, administration of rabies vaccine should be done. There are no licensed biologicals currently for PEP of domestic animals, which are not vaccinated previously. There is no reliable evidence that the sole use of vaccine will prevent the disease in these animals [140]. There is need to evaluate animals overdue for a booster vaccination on a case-by-case basis for severity of exposure, time lapse since last vaccination, number of previous vaccinations, current health status and rabies epidemiology locally. In all species of livestock, rabies infection can occur but most frequently reported species are cattle and horses [34]. Upon exposure, there is requirement of revaccination immediately followed by observation for 45 days. Unvaccinated livestock must be kept under close observation for a period of 6 months. It is suggested to euthanize animals suggestive of developing signs of rabies and head must be shipped for testing [119].

Rabies vaccines among all the vaccines can be administered uniquely pre- and post-exposure to virus. For travelers as well as veterinarians and researchers pre-exposure vaccination is appropriate, especially in regions where the disease is endemic in nature [141, 142, 143]. As it is easy to identify the event of exposure (usually a bite) and the incubation period is adequately long post-exposure immunization is possible for inducing a protective immune response. Principally, this is possible by the production of neutralizing antibodies.

Usually the immunization at post-exposure is followed by injection of immune-globulins specific for RABV either of equine or human origin. In spite of the extreme consequences of development of a disease, it is an important factor in those areas of the world which are resource-poor [144].

In human since their development more than four decades ago, concentrated, purified cell culture and embryonated egg-based rabies vaccines (CCEEVs) have proved to be safe and effective in preventing rabies. These vaccines are intended for both pre- and post-exposure prophylaxis and have been administered to millions of people worldwide [145]. Prompt administration of CCEEVs after exposure combined with proper wound management and simultaneous administration of rabies immune-globulins is almost invariably effective in preventing rabies, even after high-risk exposure [145]. Nerve tissue vaccines induce more severe adverse reactions and are less immunogenic than CCEEVs. Since 1984, WHO has recommended discontinuation of the production and use of nerve tissue vaccines and their replacement by CCEEVs.

Challenges Encountered in Rabies Vaccine Development: The greatest challenge in the industry of anti-rabies vaccine production for both humans and animals, especially in some developing countries, is little or non-availability of modern technologies in order to transit from production of nerve tissue vaccine (NTV) to the tissue culture (MTCV) [146] or sub-unit vaccines. For instance, due to presence of high content of myelin of the adult brain tissue in NTV there is a high incidence of neuroparalysis after usage of the NTV [147]. NTV is not only paralyticogenic, it is less convenient, less immunogenic, more reactogenic, less tolerable and less acceptable. In addition, more number of doses is needed and the administration of which comprise comparatively a painful procedure. MTCV however is more antigenic, acceptable, well tolerated and convenient with less reactogenicity [146].

Future Perspectives: There is a need to enhance the surveillance for RABV variants currently circulating in rabid animals particularly in wildlife. The exact prevalence of rabies in wild animals is presently lacking, which should be explored to know the role of these animals in disease transmission. The continuous monitoring of less common non-reservoir species is also important for detecting newly introduced RABV variants. More researches should be focused in the near future for the unknown aspects of pathology and pathogenesis of rabies, which would pave the way for developing suitable antiviral therapeutic strategies.

CONCLUSION AND RECOMMENDATIONS

Rabies is a viral disease that can be spread by domestic and wild animals. Many countries having the status of high-risk areas but most of the countries around the globe gained the status of rabies free territories on the basis of the results of diagnostic tests. This shows that rabies can be successfully ruled out from the high-risk areas by taking preventing measures. The advent of scientific medicine also makes rabies control possible. It is however of utmost importance to consider rabies at the very early stage along with precautionary measures. Public awareness in this regard can play major role. Strict barrier precautions are mandatory as the RABV as such is transmissible through contact of saliva with skin (broken) as well as mucous membrane. From the side of the veterinarians, it is important that they notify the appropriate authorities along with appropriate reporting of the disease though it can vary with jurisdiction. Advancement in the molecular diagnosis with the development of sequence specific RT-PCR technology has made the diagnosis very much target specific. Advanced molecular diagnostic techniques should be developed using recent biotechnological tools for early confirmatory diagnosis and differentiation of various genotypes of RABV. The following recommendations were forwarded:

- A well-coordinated one health approach is recommended for its control in both humans and animals.
- Awareness creation is mandatory concerning: rabies transmission routes, avoiding contact with wildlife and following appropriate veterinary care.
- In cases of observing any new cases, detailed molecular investigation of the virus is needed to understand the type and nature of the circulating virus.
- Local governments should initiate and maintain effective programs to ensure vaccination of all dogs, cats and ferrets and to remove strays and unwanted animals at the intersection of domestic and wild animal population. Hence, quality and protective vaccine are needed to control the disease.

REFERENCES

1. Zulu, G.C., C.T. Sabeta and L.H. Nel, 2009. Molecular epidemiology of rabies focus on domestic dogs (Canisfamiliaris) and black-backed jackals (Canis mesomelas) from northern South Africa. Virus Res., 140: 71-78.
2. Ajaya, K., 2005. Text book of preventive veterinary medicine (2nd ed). Army printing press, India.
3. Dawit, A., 1986. Traditional medicine in Ethiopia. The attempt being made to promote it for effective and better utilization SINET: Ethiopia J. Sci., 9: 6269. Addis Ababa, Ethiopia.
4. Jackson, A.C., 2003a. Neuronal apoptosis in experimental rabies: role of the route of viral entry. Neurology, 60: A102.
5. Fu, Z.F. and A.C. Jackson, 2005. Neuronal dysfunction and death in rabies virus infection. J Neurovirol, 11: 101-106.
6. WHO, 2005. WHO Expert Consultation on rabies. World Health Organ Tech. Rep. Ser., 931: 1-88, back cover.
7. Pritchard, J. and Z. Dagnatchew, 2010. Animal welfare policy and Legislative Gap Analysis. Addis Ababa, Ethiopia.
8. Deressa, A., A. Ali, M. Beyene, B. Newaye Selassie and E. Yimer, 2010. The status of rabies in Ethiopia: A retrospective record review. Ethio. J. Health Dev., 24: 127-132.
9. Knobel, D.L., S. Cleaveland, P.G. Coleman, E.M. Fevre and M.I. Meltzer, 2005. Re-evaluating the burden of rabies in Africa and Asia. Bulletin of the World Health Organization, 83: 360-368.
10. Greene, C.E. and C.E. Rupprecht, 2006. Rabies and other lyssavirus infections. In: Greene CE, editor. Infectious diseases of the dog and cat. St Louis: Elsevier Saunders, pp: 167-183.
11. Hemachudha, T., J. Laothamatas and C.E. Rupprecht, 2002. Human rabies: a disease of complex neuropathogenic mechanism and diagnostic challenges. Lancet Neurol., 1: 101-109.
12. Winkler, W.G. and K. Bogel, 1992. Control of rabies in wildlife. Sci Am., 266: 86.
13. Jackson, A.C., 2002. Pathogenesis. In: Rabies. Jackson AC and Wunner WH, editors. San Diego (CA): Academic Press, pp: 245-282.
14. Tordo, N., O. Poch, A. Ermine, G. Keith and F. Rougeon, 1986. Walking along the rabies genome: is the large G-L intergenic region a remnant gene? Proceed Nat Acad Sci., USA, 83: 3914-3918.
15. WHO, 1996. Laboratory Techniques in Rabies, Fourth Edition, Meslin F.-X., Kaplan M.M. & Koprowski H., eds. WHO, Geneva, Switzerland.
16. Baer, G.M., 1991. The Natural History of Rabies, Second Edition. CRC Press, Boca Raton, Florida, USA, pp: 620.
17. Bourhy, H., B. Kissi and N. Tordo, 1993. Molecular diversity of the lyssavirus genus. Virology, 194: 70-81. 17.
18. Hooper, P.T., R.A. Lunt, A.R. Gould, H. Samaratunga, A.D. Hyatt, L.J. Gleeson, B.J. Rodwell, C.E. Rupprecht, J.S. Smith and P.K. Murray, 1997. A new lyssavirus the first endemic rabies-related virus recognized in Australia. Bull. Inst. Pasteur, 95: 209-218.
19. Freuling, C.M., M. Beer, F.J. Conraths, S. Finke, B. Hoffmann, B. Keller and T. Muller, 2011. Novel lyssavirus in Natterer's bat, Germany. Emerg Infect Dis., 17: 1519-1522.
20. Marston, D., D.L. Horton, C. Ngeleja, K. Hampson, L.M. McElhinney, A.C. Banyard and T. Lembo, 2012. Ikoma lyssavirus, highly divergent novel lyssavirus in African civet. Emerg Infect Dis., 18: 664-667.
21. Drew, W.L., 2004. "Chapter 41: Rabies". In Ryan KJ, Ray CG (editors). Sherris Medical Microbiology (4th ed.). McGraw Hill, pp: 597-600.
22. Albertini, A.A., R.W. Ruigrok and D. Blondel, 2011. Rabies virus transcription and replication. Adv. Virus Res., 79: 1-22.
23. Albertini, A.A., G. Schoehn, W. Weissenhorn and R.W. Ruigrok, 2008. Structural aspects of rabies virus replication. Cell Mol Life Sci., 65(2): 282-294.
24. CDC, 2011. The Rabies Virus. http://www.cdc.gov/rabies/transmission/ virus.html. Accessed on May 24, 2021.
25. Gaudin, Y., 2000. Rabies virus-induced membrane fusion pathway. Journal of Cell Biology, 150: 601-611.
26. http://viralzone.expasy.org/all_by_species/2.html. Accessed on 16 June, 2021
27. Arnheiter, H., N.L. Davis, G. Wertz, M. Schubert and R.A. Lazzarini, 1985. Role of the Nucleocapsid Protein in Regulating Vesicular Stomatitis-Virus Rna-Synthesis. Cell, 41: 259-267.
28. Lahaye, X., A. Vidy, C. Pomier, L. Obiang and F. Harper, 2009. Functional characterization of Negri bodies (NBs) in rabies virus-infected cells: Evidence that NBs are sites of viral transcription and replication. J. Virol., 83: 7948-7958.
29. Lackay, S.N., Y. Kuang and Z.F. Fu, 2008. Rabies in small animals. Vet Clin North Am Small Anim Pract 38: 851-861, ix.

30. Morimoto, K., M. Patel, S. Corisdeo, D.C. Hooper and Z.F. Fu, 1996. Characterization of a unique variant of bat rabies virus responsible for newly emerging human cases in orth America. Proceedings of the National Academy of Sciences of the United States of America 93: 5653-5658.
31. Gruzdev, K.N., 2008. The rabies situation in Central Asia. Dev Biol (Basel)., 131: 37-42.
32. Seimenis, A., 2008. The rabies situation in the Middle East. Dev Biol (Basel)., 131: 43-53.
33. Blancou, J., 2008. The control of rabies in Eurasia: overview, history and background. Dev Biol (Basel), 131: 3-15.
34. Blanton, J.D., D. Palmer, K.A. Christian and C.E. Rupprecht, 2008. Rabies surveillance in the United States during 2007. J. Am. Vet. Med. Assoc., 233: 884-897.
35. Wilkins, P.A. and F.D. Piero, 2007. Rabies. In: Sellon, DC and Long, MT, editors. Equine infectious diseases. Philadelphia (PA): Saunders, Elsevier Inc; Chapter, 19: 185-191.
36. Hanna, J.N., I.K. Carney, G.A. Smith, A.E. Tannenberg, J.E. Deverill, J.A. Botha, I.L. Serafin, B.J. Harrower, P.F. Fitzpatrick and J.W. Searle, 2000. Australian bat lyssavirus infection: a second human case, with a long incubation period. Med. J. Aust., 172(12): 597-599.
37. Francis, J.R., C. Nourse, V.L. Vaska, S. Calvert, J.A. Northill, B. McCall and A.C. Mattke, 2014. Australian bat lyssavirus in a child: the first reported case. Pediatrics, 133(4): e1063-e1067.
38. Finnegan, C.J., S.M.N. Brookes Johnson, J. Smith and K.L. Mansfield, 2002. Rabies in North America and Europe. J R Soc. Med., 95: 9-13.
39. Irsara, A., A. Ruatti, G. Gagliardi, Z. Orfei, L. Bellani and A., Mantovani, 1982. Control of wildlife rabies in North-Eastern Italy. Comp Immunol Microbiol Infect Dis., 5: 327-335.
40. Sillerozubiri, C., A.A. King and D.W. Macdonald, 1996. Rabies and mortality in Ethiopian wolves (*Canis simensis*). Journal of Wildlife Diseases, 32: 80-86.
41. Constantine, D.G., 1979. Updated List of Rabies-Infected Bats in North- America. Journal of Wildlife Diseases, 15: 347-349.
42. Krebs, J.W., C.E. Rupprecht and J.E. Childs, 2000. Rabies surveillance in the United States during 1999. Journal of the American Veterinary Medical Association, 217: 1799-1811.
43. Malerczyk, C., L. De Tora and D. Gniel, 2011. Imported human rabies cases in Europe, the United States and Japan, 1990 to 2010. Journal of Travel Medicine, 18: 402-407.
44. http://www.integratedveterinarypathologybyalexandrabrower.com/uploads/1/4/1/0/14103113/1353812031.jpg. Accessed on 18 June, 2021.
45. Franka, R., X. Wu, F.R. Jackson, A. Velasco-Villa, D.P. Palmer, H. Henderson, W. Hayat, D.B. Green, J.D. Blanton, L. Greenberg and C.E. Rupprecht, 2009. Rabies virus pathogenesis in relationship to intervention with inactivated and attenuated rabies vaccines. PubMed., 27(51): 7149-7155.
46. Benmansour, A., H. Leblois, P. Coulon, C. Tuffereau and Y. Gaudin, 1991. Antigenicity of rabies virus glycoprotein. J. Virol., 65: 4198-4203.
47. Ito, N., M. Takayama, K. Yamada, M. Sugiyama and N. Minamoto, 2001. Rescueof rabies virus from cloned cDNA and identification of the pathogenicityrelated gene: glycoprotein gene is associated with virulence for adult mice. J. Virol., 75: 9121-9128.
48. Lentz, T.L., T.G. Burrage, A.L. Smith, J. Crick and G.H. Tignor, 1982. Is the Acetylcholine- Receptor a Rabies Virus Receptor. Science, 215: 182-184.
49. Thoulouze, M.I., M. Lafage, M. Schachner, U. Hartmann, H. Cremer and M. Lafon, 1998. The neural cell adhesion molecule is a receptor for rabies virus. J. Virol., 72: 7181-7190.
50. Thoulouze, M.I., H.D. Foley, J.P. McGettigan, M.J. Schnell and B. Dietzschold, 2000. Reinvestigation of the role of the rabies virus glycoprotein in viral pathogenesis using a reverse genetics approach. Journal of Neurovirology, 6: 373-381.
51. Shankar, V., B. Dietzschold and H. Koprowski, 1991. Direct entry of rabies virus into the central nervous system without prior local replication. J. Virol., 65: 2736-2738.
52. Tsiang, H., P.E. Ceccaldi and E. Lycke, 1991. Rabies virus infection and transport in human sensory dorsal root ganglia neurons. J. Gen. Virol., 72(Pt 5): 1191-1194.
53. Wang, Z.W., L. Sarmento, Y.H. Wang, X.Q. Li, V. Dhingra and T. Tseggai, 2005. Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. Journal of Virology, 79: 12554-12565.

54. Sarmento, L., T. Tseggai, V. Dhingra and Z.F. Fu, 2006. Rabies virus-induced apoptosis involves caspase-dependent and caspase-independent pathways. Virus Research, 121: 144-151.
55. Zhang, G.Q., H.L. Wang, F. Mahmood and Z.F. Fu, 2013. Rabies virus glycoprotein is an important determinant for the induction of innate immune responses and the pathogenic mechanisms. Veterinary Microbiology, 162: 601-613.
56. Fabis, M.J., T.W. Phares, R.B. Kean, H. Koprowski and D.C. Hooper, 2008. Bloodbrain barrier changes and cell invasion differ between therapeutic immune clearance of neurotrophic virus and CNS autoimmunity. Proceedings of the National Academy of Sciences of the United States of America, 105: 15511-15516.
57. Kuang, Y., S.N. Lackay, L. Zhao and Z.F. Fu, 2009. Role of chemokines in the enhancement of BBB permeability and inflammatory infiltration after rabies virus infection. Virus Research, 144: 18-26.
58. Morimoto, K., D.C. Hooper, S. Spitsin, H. Koprowski and B. Dietzschold, 1999. Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. J. Virol., 73: 510-518.
59. Yan, X., M. Prosniak, M.T. Curtis, M.L. Weiss and M. Faber, 2001. Silver- haired bat rabies virus variant does not induce apoptosis in the brain of experimentally infected mice. J. Neurovirol., 7: 518-527.
60. Niu, X., H. Wang and Z.F. Fu, 2011. Role of chemokines in rabies pathogenesis and protection. Adv Virus Res., 79: 73-89.
61. Shoji, Y., S. Inoue, K. Nakamichi, I. Kurane, T. Sakai and K. Morimoto, 2004. Generation and characterization of P gene-deficient rabies virus. Virology, 318: 295-305.
62. Megret, F., C. Prehaud, M. Lafage, Batejat and N. Escriou, 2005. Immunopotentiation of the antibody response against influenza HA with apoptotic bodies generated by rabies virus G-ERA protein-driven apoptosis. Vaccine, 23: 5342-5350.
63. Phares, T.W., R .B. Kean, T. Mikheeva and D.C. Hooper, 2006. Regional differences in blood-brain barrier permeability changes and inflammation in the apathogenic clearance of virus from the central nervous system. J. Immunol., 176: 7666-7675.
64. Faber, M., J. Li, R.B. Kean, D.C. Hooper, K.R. Alugupalli and B. Dietzschold, 2009. Effective preexposure and postexposure prophylaxis of rabies with a highly attenuated recombinant rabies virus. Proc Natl Acad Sci. USA, 106: 11300- 11305.
65. Dietzschold, B., W.H. Wunner, T.J. Wiktor, A.D. Lopes, M. Lafon, C.L. Smith and H. Koprowski, 1983. Characterization of an antigenic determinant of the glycoprotein that correlates with pathogenicity of rabies virus. Proc Natl Acad Sci. USA, 80: 70-74.
66. Marsh, M. and A. Helenius, 1989. Virus entry into animal cells. Adv Virus Res., 36: 107-151.
67. Gaudin, Y., C. Tuffereau, D. Segretain, M. Knossow and A. Flamand, 1991. Reversible conformational changes and fusion activity of rabies virus glycoprotein. J. Virol., 65: 4853-4859.
68. Faber, M., M.L. Faber, A. Papaneri, M. Bette, E. Weihe, B. Dietzschold, M.J. Schnell, 2005. A single amino acid change in rabies virus glycoprotein increases virus spread and enhances virus pathogenicity. J Virol., 79: 14141-14148.
69. Faber, M., R. Pulmanausahakul, K. Nagao, M. Prosniak, A.B. Rice, H. Koprowski, M.J. Schnell and B. Dietzschold, 2004. Identification of viral genomic elements responsible for rabies virus neuroinvasiveness. Proc. Natl. Acad. Sci. USA., 101: 16328-16332.
70. Schnell, M.J., T. Mebatsion and K.K. Conzelmann, 1994. Infectious rabies viruses from cloned cDNA. EMBO J., 13: 4195-4203.
71. Iwata, M., S. Komori, T. Unno, N. Minamoto and H. Ohashi, 1999. Modification of membrane currents in mouse neuroblastoma cells following infection with rabies virus. Br J Pharmacol., 126: 1691-1698.
72. Dietzschold, B., T.J. Wiktor, J.Q. Trojanowski, R.I. Macfarlan, W.H. Wunner, M.J. Torres-Anjel and H.Koprowski, 1985. Differences in cell-to-cell spread of pathogenic and apathogenic rabies virus *in vivo* and *in vitro*. J. Virol., 56: 12-18.
73. Coulon, P., C. Derbin, P. Kucera, F. Lafay, C. Prehaud and A.Flamand, 1989. Invasion of the peripheral nervous systems of adult mice by the CVS strain of rabies virus and its avirulent derivative AvO1. J. Virol., 63: 3550-3554.
74. Etessami, R., K.K. Conzelmann, B. Fadai-Ghotbi, B. Natelson, H. Tsiang and P.E. Ceccaldi, 2000. Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an in vitro and in vivo study. J Gen Virol., 81: 2147-2153.

75. Pulmanausahakul, R., J. Li, M.J. Schnell and B. Dietzschold, 2008. The glycoprotein and the matrix protein of rabies virus affect pathogenicity by regulating viral replication and facilitating cell-to-cell spread. J. Virol., 82: 2330-2338.
76. Mebatsion, T., F. Weiland and K.K. Conzelmann, 1999. Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. J. Virol., 73: 242-250.
77. Tan, GS., M.A. Preuss, J.C. Williams and M.J. Schnell, 2007. The dynein light chain 8 binding motif of rabies virus phosphoprotein promotes efficient viral transcription. Proc. Natl. Acad Sci. USA, 104:7229-7234.
78. https://europepmc.org/articles/PMC6899062/bin/nihms-1059081-f0001.jpg, Accessed on July 04, 2021
79. Fooks, A.R., N. Johnson, C.M. Freuling, P.R. Wakely, A.C. Banyard, L.M. McElhinney, R. Franka, X. Wu, F.R. Jackson, A. Velasco-Villa, D.P. Palmer and H. Henderson, 2009. Rabies virus pathogenesis in relationship to intervention with inactivated and attenuated rabies vaccines. Vaccine, 27: 7149-7155.
80. OIE, 2013. Manual of diagnostic tests and vaccines for terrestrial animals. CHAPTER 2.1.13.Rabieshttp://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.01.13_RABIES.pd.
81. Meslin, F.X., D.B. Fishbein and H.C. Matter, 1994. Rationale and prospects for rabies elimination in developing countries. Curr. Topics Microbiol. Immunol., 187: 1-26.
82. WHO, 1999. WHO. World Survey of rabies No. 32 for year 1998.
83. Bingham, J. and M. Van Der Merwe, 2002. Distribution of rabies antigen in infected brain material: determining the reliability of different regions of the brain for the rabies fluorescent antibody test. J. Virol. Methods, 101: 85-94.
84. Barrat, J. and J. Blancou, 1988. Technique simplifiée de prélèvement, de conditionnement et d'expédition de matière cérébrale pour le diagnostic de rage. Doc. WHO/Rab. Res./88.27.
85. Montano hirose, J.A., H. Bourhy and P. Sureau, 1991. Retro-orbital route for brain specimen collection for rabies diagnosis. Vet. Rec., 129: 291-292.
86. Bourhy, H. and P. Sureau, 1991. Méthodes de laboratoire pour le diagnostic de la rage; Metodos de laboratorio para el diagnostics de la rabia; Laboratory methods for rabies diagnosis. Commission des Laboratoires de référence et d'Expertise de I'Institut Pasteur, Paris, France, 197.
87. Jackson, A.C., 2010. Rabies pathogenesis update. Rev Pan-Amaz Saude, 1(1): 167-172.
88. Hemachudha, T., G. Ugolini, S. Wacharapluesadee, W. Sungkarat, S.Shuangshoti and J. Laothamatas, 2013. Human rabies: neuropathogenesis, diagnosis and management. Lancet Neurol., 12(5): 498-513.
89. Blanton, J.D., D. Palmer, K.A. Christian, C.E. Rupprecht, 2010. Rabies surveillance in the United States during 2009. J. Am. Vet. Med. Assoc., 237(6): 646-657.
90. Campbell, J.B., K.M. Charlton, editors, 1988. Rabies. Boston (MA): Kluwer Academic Publications.
91. Suraweera, W., S.K. Morris, R. Kumar, D.A. Warrell, M.J. Warrell and P. Jha, 2012. Deaths from symptomatically identifiable furious rabies in India: A nationally representative mortality survey. PLoS Negl. Trop Dis., 6(10): e1847.
92. Udow, S.J., R.A. Marrie and A.C. Jackson, 2013. Clinical features of dog- and bat-acquired rabies in humans. Clin Infect Dis., 57(5): 689-696.
93. Aubert, M.F.A., 1982. Sensibilité et fidélité du diagnostic de rage au laboratoire. Comp. Immunol. Microbiol. Infect. Dis., 5: 369-376.
94. http://virology-online.com/general/rabiesdfa.gif. Accessed on 29 June, 2016.
95. Barnard, B.J.H. and S.F. Voges, 1982. A simple technique for the diagnosis of rabies in formalin-preserved brain. Ondersterpoort J. Vet. Res., 49: 193-194.
96. Barrat, J., 1992. Experimental diagnosis of rabies. Adaptations to field and tropical conditions. Proceedings of the International Conference on Epidemiology, Control and Prevention of Rabies in Eastern and Southern Africa. Lusaka, Zambia, 2-5 June 1992, pp: 72-83.
97. Rudd, R.J. and C.V. Trimachi, 1987. Comparison of sensitivity of BHK-21 and murine neuroblastoma cells in the isolation of a street strain rabies virus. J. Clin. Microbiol., 25: 145-168.
98. Barrat, J., M.J. Barrat, M. Picard and M.F.A. Aubert, 1986. Diagnostic de la rage sur culture cellulaire, comparaison des résultats de l'inoculation au neuroblastome murin et de l'inoculation à la souris. Comp. Immunol. Microbiol. Infect. Dis., 11: 207-214.
99. Orciari, L.A., C.E. Rupprecht and J. Versalovic, 2011. Rabies. In: Manual of clinical microbiology, 10th ed. Washington DC, ASM Press, pp: 1470-1478.
100. WHO, 1985. Expert committee on biological standards. thirty-fifth report World Health Organisation Technical Report Series No. 725. WHO, Geneva, Switzerland.

101. Cliquet, F., L. Sagne, J.L. Schereffer and M.F.A. Aubert, 2000. ELISA tests for rabies antibody titration in orally vaccinated foxes sampled in the fields. Vaccine, 18: 3272-3279.
102. Wacharapluesadee, S., P. Phumesin, P. Supavonwong, P. Khawplod, N. Intarut and T. Hemachudha, 2011. Comparative detection of rabies RNA by NASBA, real-time PCR and conventional PCR. J. Virol. Methods, 175(2): 278-282.
103. Meng, S., W. Lv, J. Wu, J. Wang, G. Xu and J. Yan, 2013. Comparison of three strategies for sequencing rabies viral G gene. J. Appl. Virol., 2: 1.
104. PicardMeyer, E., V. Bruyere, J. Barrat, E. Tissot, M.J. Barrat and F. Cliquet, 2004. Development of a hemi-nested RT-PCR method for the specific determination of European bat lyssa virus 1 comparison with other rabies diagnostic methods. Vaccine, 22: 1921-1929.
105. Coertse, J., J. Weyer, L.H. Nel and W. Merkotter, 2010. Improved PCR methods for detection of African rabies and rabies-related lyssaviruses. J. Clin. Microbiol., 48(11): 3949-3955.
106. Aravindh Babu, R.P., S. Manoharan and P. Ramadass, 2014. Diagnostic evaluation of RT-PCR-ELISA for the detection of rabies virus. Indian J. Virol., 25(1):120-124.
107. Babu, R.A., S. Manoharan, P. Ramadass and N.D.J. Chandran, 2012. Evaluation of RT-PCR assay for routine laboratory diagnosis of Rabies in post mortem brain samples from different species of animals. Indian J. Virol., 23(3): 392-396.
108. David, D., 2012. Role of the RT-PCR method in ante-mortem and post-mortem rabies diagnosis. Indian J. Med. Res., 135(6): 809.
109. Dandale, M., C.K. Singh, V. Ramneek, D. Deka, K. Bansal and N.K. Sood, 2013. Sensitivity comparison of nested RT-PCR and TaqMan real time PCR for intravitam diagnosis of rabies in animals from urine samples. Vet World, 6(4): 189-192.
110. Mani, R.S., S.N. Madhusudana, A. Mahadevan, V. Reddy, A.Y. Belludi and S.K. Shankar, 2014. Utility of real-time Taqman PCR for antemortem and postmortem diagnosis of human rabies. J. Med. Virol., 86(10): 1804-1812.
111. De Benedictis, P., C. De Battisti, S. Marciano, F. Mutinelli, I. Capua and G. Cattoli, 2013. Pyrosequencing of the rabies virus glycoprotein gene to demonstrate absence of vaccine-associated rabies cases following oral vaccination. Veterinary J., 195(3): 388-390.
112. Lung, O., S. Nadin-Davis, M. Fisher, A. Erickson, M.K. Knowles, T. Furukawa-Stoffer and A. Ambagala, 2013. Microarray for identification of the chiropteran host species of rabies virus in Canada. Microarrays, 2(2): 153-169.
113. Bourhy, H., B. Kissi, L. Audry, M. Smreczak, M. Sadkowska Todys, K. Kulonen, N. Tordo, J.F. Zmudzinski and E.C. Holmes, 1999. Ecology and evolution of rabies virus in Europe. J. Gen. Virol., 80(10): 2545-2557.
114. Dupuis, M., S. Brunt, K. Appler, A. Davis and R. Rudd, 2015. Comparison of automated quantitative reverse transcription-PCR and direct fluorescent-antibody detection for routine rabies diagnosis in the United States. J Clin Microbiol., 53(9): 2983-2989.
115. Suin, V., F. Nazé, A. Francart, S. Lamoral, S. De Craeye, M. Kalai and S. Van Gucht, 2014. A two-step lyssavirus real-time polymerase chain reaction using degenerate primers with superior sensitivity to the fluorescent antigen test. Biomed Res. Int., 2014: 256175.
116. Rupprecht, C.E., J. Barrett, D. Briggs, F. Cliquet, A.R. Fooks, B. Lumlertdacha, F.X. Meslin, T. Muler, L.H. Nel and C. Schneider, 2008. Can rabies be eradicated? Dev. Biol. (Basel), 131: 95-121.
117. Cliquet, F. and M. Aubert, 2004. Elimination of terrestrial rabies in Western European countries. Dev Biol (Basel), 119: 185-204.
118. Nadin-Davis, S.A. and C. Fehlner-Gardiner, 2008. Lyssaviruses-current trends. Adv. Virus Res., 71: 207-250.
119. Green, S.L., 1993. Equine rabies. Vet Clin North Am Equine Pract., 9: 337-347.
120. Weese, J.S., 2002. A review of equine zoonotic diseases: risks in veterinary medicine. Proc Annual Convention AAEP., 48: 362-369.
121. Lau, C.L. and N. Hohl, 2013. Immunogenicity of a modified intradermal pre-exposure rabies vaccination schedule using a purified chick embryo cell vaccine: an observational study. Travel Med Infect Dis., 11(6): 427-430.
122. Stading, B.R., J.E. Osorio, A. Velasco-Villa, M. Smotherman, B. Kingstad Bakke and T.E. Rocke, 2016. Infectivity of attenuated poxvirus vaccine vectors and immunogenicity of a raccoonpox vectored rabiesvaccine in the Brazilian free-tailed bat (Tadarida brasiliensis). Vaccine, 34(44): 5352-5358.

123. Liu, Y., H.P. Zhang, S.F. Zhang, J.X. Wang, H.N. Zhou, F. Zhang, Y.M. Wang, L. Ma, N. Li and R.L. Hu, 2016. Rabies outbreaks and vaccination in domestic camels and cattle in Northwest China. PLoS Negl Trop Dis., 10(9): e0004890.
124. Yang, D.K., H.H. Kim, K.W. Lee and J.Y. Song, 2013. The present and future of rabies vaccine in animals. Clin Exp Vaccine Res., 2: 19-25.
125. Ohara, S., S. Sato, K. Oyama, K.I. Tsutsui and T. Iijima, 2013. Rabies virus vector transgene expression level and cytotoxicity improv ement induced by deletion of glycoprotein gene. PloS One, 8(11): e80245.
126. Montaner, A.D., A. DeNichilo, J.M. Rodriguez, A. HernandoInsua, J. Fló, R.A. Lopez, Sierra, C. Paolazzi, O. Larghi and D.L. Horn, 2012. IMT504: A new and potent adjuvant for rabies vaccines permitting significant dose sparing. World J. Vaccines., 2: 182.
127. Dhama, K., S. Chakraborty, Mahima, M.Y. Wani, A.K. Verma, R. Deb, R. Tiwari and S. Kapoor, 2013. Novel and emerging therapies safeguarding health of humans and their companion animals: A review. Pak J. Biol. Sci., 16(3): 101-111.
128. Muller, T.F., P. Schuster, A.C. Vos, T. Selhorst, U.D. Wenzel and A.M. Neubert 2001. Effect of maternal immunity on the immune response to oral vaccination against rabies in young foxes. Am. J. Vet. Res., 62: 1154-1158.
129. Cliquet, F., J.P. Gurbuxani, H.K. Pradhan, B. Pattnaik, S.S. Patil, A. Regnault, H. Begouen, A.L. Guiot, R. Sood and P. Mahl, 2007. The safety and efficacy of the oral rabies vaccine SAG2 in Indian stray dogs. Vaccine, 25: 3409-3418.
130. Xiang, Z.Q., G.P. Gao, A. Reyes-Sandoval, Y. Li, J.M. Wilson and H.C. Ertl, 2003. Oral vaccination of mice with adenoviral vectors is not impaired by preexisting immunity to the vaccine carrier. J. Virol., 77: 10780-10789.
131. Kieny, M.P., R. Lathe, R. Drillien, D. Spehner and S. Skory, 1984. Expression of rabies virus glycoprotein from a recombinant vaccinia virus. Nature, 312: 163-166.
132. Brochier, B., M.P. Kieny, F. Costy, P. Coppens, B. Bauduin, J.P. Lecocq, B. Languet, G. Chappuis, P. Desmetre and K. Afiademanyo, 1991. Large-scale eradication of rabies using recombinant vaccinia rabies vaccine. Nature, 354: 520-522.
133. Liu, X., Y. Yang, Z. Sun, J. Chen, J. Ai, C. Dun, Z.F. Fu, X. Niu and X. Guo, 2014. A recombinant rabies virus encoding two copies of the glycoprotein gene confers protection in dogs against a virulent challenge. PloS One, 9(2): e87105.
134. Xue, X.H., X.X. Zheng, H.L. Wang, J.Z. Ma, L. Li, W.W. Gai, T.C. Wang, S.T. Yang and X.Z. Xia, 2014. An inactivated recombinant rabies CVS-11 virus expressing two copies of the glycoprotein elicits a higher level of neutralizing antibodies and provides better protection in mice. Virus Genes, 48(3): 411-420.
135. Mills, D., 2013. Intradermal pre-exposure rabies vaccination. Promises and pitfalls. Travel Med. Inf. Dis., 11(6): 335-336.
136. Joob, S., 2013. Rabies vaccination in special scenarios: A review. Adv Trop Med Pub Health Int., 3(1): 1-4.
137. Shah, M.A.A., S.U. Khan, Z. Ali, H. Yang, K. Liu and L. Mao, 2014. Applications of nanoparticles for DNA based rabies vaccine. J. Nanosci Nanotechnol., 14(1): 881-891.
138. McGettigan, J.P., F. David, M.D. Figueiredo, J. Minke, T. Mebatsion and M.J. Schnell, 2014. Safety and serological response to a matrix gene-deleted rabies virus-based vaccine vector in dogs. Vaccine, 32(15): 1716-1719.
139. Dorfmeier, C.L., A.G. Lytle, A.L. Dunkel, A. Gatt and J.P. McGettigan, 2012. Protective vaccine-induced CD4+ T cell-independent B cell responses against rabies infection. J. Virol., 86(21): 11533-11540.
140. Hanlon, C.A., M. Niezgoda, P. Morrill and C.E. Rupprecht, 2002. Oral efficacy of an attenuated rabies virus vaccine in skunks and raccoons. Journal of Wildlife Diseases, 38: 420-427.
141. Carrara, P., P. Parola, P. Brouqui and P. Gautret, 2013. Imported human rabies cases worldwide, 1990-2012. PLoS Negl Trop Dis., 7(5): e2209.
142. Permpalung, N., S. Wongrakpanich, S. Korpaisarn, P. Tanratana and J. Angsanakul, 2013. Trend of human rabies prophylaxis in developing countries: Toward optimal rabies immunization. Vaccine, 31(38): 4079-4083.
143. Weant, K.A. and S.N. Baker, 2013. Review of human rabies prophylaxis and treatment. Crit Care Nurs. Clin North Am., 25(2): 225-242.

144. Joseph, J., N. Sangeetha, A.M. Khan and O.P. Rajoura, 2013. Determinants of delay in initiating post-exposure prophylaxis for rabies prevention among animal bite cases: Hospital based study. Vaccine, 32(1): 74-77.
145. WHO, 2010. Position paper on rabies vaccines. Weekly Epidemiological Record, 85: 309-320.
146. Ghosh, T.K., 2005. Bihar Pedicon -Conference Abstracts. Pediatric On call. 10. Greene CE, Rupprecht CE. 2006. Rabies and other lyssavirus infections. In: Greene CE, editor. Infectious diseases of the dog and cat. St Louis: Elsevier Saunders, pp: 167-183.
147. Acha, P.N., 1981. A Review of Rabies Prevention and Control in the Americas, 1970- 1980: Overall status of rabies. Bull Off Int Epiz., 93: 9-52.